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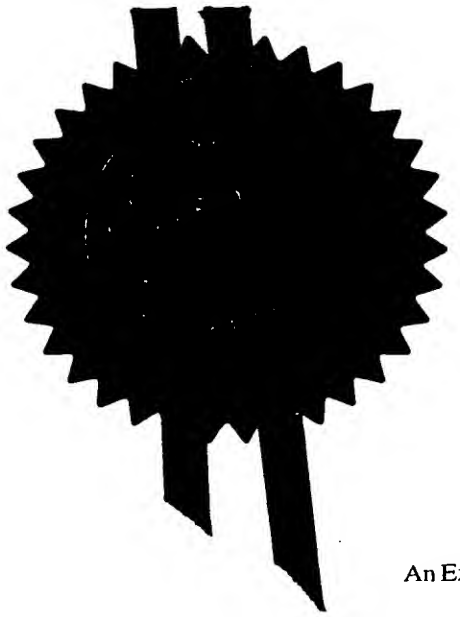

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1. Your Reference — RMT/MG/PG3672

2. Patent application number *(The Patent office will fill in this part)* — 9912534.6

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

GLAXO GROUP LIMITED
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UB6 0NN, GB

Patents ADP number *(if you know it)* 05553680002.

If the applicant is a corporate body, give the country/state of its corporation

4 Title of the invention — NOVEL PROGNOSTIC SURROGATE

5 Name of your agent *(if you know one)*

RACHEL M. THORNLEY
(SEE CONTINUATION SHEET)

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

GLAXO WELLCOME PLC
GLAXO WELLCOME HOUSE, BERKELEY AVENUE
GREENFORD, MIDDLESEX
UB6 ONN, GB

Patents ADP number *(if you know it)* 07062540001.

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Country	Priority application number *(if you know it)*	Date of Filing (day / month / year)

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Request for preliminary examination and search *(Patent Form 9/77)*	
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Any other documents *(please specify)*	

11. **I/We request the grant of a patent on the basis of this application**

Signature **Rachel M. Thornley** **28 May, 1999**

AGENT FOR THE APPLICANTS

12. Name and daytime telephone number of person to contact in the United Kingdom

Fiona Longstaff

0181-966 8234

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Novel Prognostic Surrogate

This invention relates to a novel method for prognosis of a patient with a respiratory disease, specifically chronic obstructive pulmonary disease.

Chronic obstructive pulmonary disease (COPD) is a disease characterised by irreversible airflow obstruction and a decline in the lung function parameter FEV1 that is more rapid than normal. The disease has two major aspects of pathology, namely chronic bronchitis, characterised by mucus hypersecretion from the conducting airways, and emphysema, characterised by destructive changes in the alveoli.

Currently a number of pharmaceutical substances are indicated for or have been shown to be useful in treating the symptoms of COPD, including salmeterol xinafoate, fluticasone propionate and ipratropium bromide. (2R,3R,4S,5R)-2-[6-Amino-2-(1S-hydroxymethyl-2-phenyl-ethylamino)-purin-9-yl]-5-(2-ethyl-2H-tetrazol-5-yl)-tetrahydro-furan-3,4-diol is also of development interest in the treatment of COPD. However there is considerable interest in evaluating the extent, if at all, these medicines are disease modifying i.e. affect the overall progression of the disease either in terms of symptom severity or exacerbation severity.

Additionally many of the symptoms of COPD are shared by other respiratory diseases such as asthma, bronchitis, pulmonary fibrosis and tuberculosis. Accordingly COPD is considered to be a poorly diagnosed disease and due to this fact a great number of patients are denied medicine that could be of benefit to them. In addition, there is a need for new medicines that will be more effective than current medicines. In view of the economic impact of COPD there is considerable incentive for drug discovery in this area.

The presenting symptoms for COPD are breathlessness accompanied by a decline in FEV1. Chronic bronchitis can be diagnosed by asking the patient whether they have a "productive cough" i.e. one that yields purulent sputum. Patients are traditionally treated with bronchodilators or steroids and examined by spirometry for reversibility of airflow obstruction. If reversibility is less than 15%, and particularly if they have a long history of smoking, then they would be classified as COPD patients.

The ATS (American Thoracic Society) criteria for diagnosing COPD are as follows:

FEV1/FVC ratio<0.7

FEV1<70% predicted, <15% reversibility to inhaled B2 agonist

PLUS:

2 week oral prednisolone trial - less than 15% reversibility in FEV1

Smoking history

Excluding alpha-1 AT deficiency (by blood test)

Non-atopic (skin tests) and no history of atopy

Stable: without exacerbation for at least 6 weeks

No history of childhood asthma

There is a need in the art to identify a reliable and straightforward indicator of the COPD disease state (for example, a surrogate marker) both in order to reliably distinguish the symptoms of COPD from those of the above mentioned respiratory diseases and to evaluate disease severity and progression, and response to medicine.

Elevated levels of cytokeratin 19 fragments have been detected in the broncheoalveolar lavage fluid of patients with chronic inflammatory lung disease and this observation was suggested as a marker of bronchial epithelial injury (Nakamura, H. et al., 1997: Am. J. Resp. Crit. Care Med. **155**, 1217-1221).

However, no attempt was made to correlate levels of this marker with lung function (e.g. FEV1).

The inventors of the present invention have surprisingly identified a hitherto unappreciated correlation between the concentration of soluble E-cadherin in blood serum and urine in a patient and the severity of COPD as measured by a reduction in the patient's FEV1.

FEV1 is the volume of air expelled from the lungs in one second, starting from a position of maximum inspiration and with the subject making maximum effort. FEV1% is the FEV1 expressed as a percentage of the forced vital capacity (FVC). The FVC is the total volume of air expelled from the lungs from a position of maximum inspiration with the subject making maximum effort.

FEV1 may be measured using a spirometer to measure the volume of air expired in the first second of exhalation.

E-cadherin is a member of the calcium dependent adhesion molecule superfamily and is expressed in epithelia, including those of the lung, gut and skin. It has a major role in controlling epithelial intercellular adhesion since it influences the formation of all epithelial intercellular junctions. Adhesion is mediated by interaction between extracellular domains of E-cadherin dimers on adjacent cells. In the adherens junction, cadherin dimers assemble in a zipper-like manner increasing the adhesive strength. In certain epithelial hyperproliferative conditions, there is some shedding of E-cadherin extracellular domains as soluble fragments, (sE-cadherin). The concentration of sE-cadherin in the circulation has been shown to be increased in patients with certain tumours and also to correlate with the PASI score (measure of disease severity) of psoriasis patients.

Concentration of E-cadherin in the blood serum or urine may be determined using a specific ELISA. Using this assay, the inventors have shown a direct and inverse linear correlation between actual FEV1 in COPD patients (as a percentage of predicted FEV1) and sE-cadherin levels in serum and urine respectively.

The results of a trial demonstrating these correlations are described in Example 1 and shown in Figures 1 and 2.

Thus the concentration of soluble E-cadherin in blood serum and urine is a molecular indicator for COPD which is capable of reporting its severity without recourse to evaluating any symptom except reduction in a patient's FEV1 .

The predicted (normal) FEV1 of a patient may be calculated by the methods determined by Morris JF et al 1971: Am Rev Resp Dis **103,** 57-67 based on given height and age. The values are influenced by age, sex and height.

A patient already diagnosed as having COPD can be assayed for disease severity at a time point by comparison of his concentration of soluble E-cadherin in blood serum or urine at that time point with the indicator of severity shown in Figures 1 and 2.

Progression of COPD disease may be evaluated by monitoring the concentration of soluble E-cadherin in blood serum or urine with time.

It will be appreciated that either the concentration of soluble E-cadherin in blood serum or urine may be measured for the prognosis, however the recordal of both measurements will be confirmatory. The strength of the confirmation is emphasised by the inverse correlation between the two measurements as shown in Figures 1 and 2.

It will be appreciated that a particular and unique benefit of the invention is the ease of prognosis which may be performed requiring only a simple blood or urine sample.

Thus, according to the invention, we provide a method of determining the severity of COPD in a patient which comprises measuring the concentration of soluble E-cadherin in the patient's urine and determining the extent of severity by reference to a correlation graph as shown in Figure 1.

We also provide a method of determining the severity of COPD in a patient which comprises measuring the concentration of soluble E-cadherin in the patient's blood serum and determining the extent of severity by reference to a correlation graph as shown in Figure 2.

For greater confidence, the method may comprise measuring the concentration of soluble E-cadherin in the patient's blood serum and urine and determining the extent of severity by reference to a correlation graph for each as shown in Figures 1 and 2.

We also provide use of soluble E-cadherin in the manufacture of a prognostic product for determination of COPD disease severity in a patient.

As a further aspect of the invention we provide a method of determining the responsiveness of a patient with COPD to therapy which comprises monitoring the concentration of soluble E-cadherin in the blood serum of the patient with time and determining the rate of change of extent of progression of the disease by reference to a correlation graph as shown in Figure 1.

We also provide a method of determining the responsiveness of a patient with COPD to therapy which comprises monitoring the concentration of soluble E-cadherin in the urine of the patient with time and determining the rate of change of extent of progression of the disease by reference to a correlation graph as shown in Figure 2.

For greater confidence, the method may comprise monitoring the concentration of soluble E-cadherin in the urine and blood serum of the patient with time and determining the rate of change of extent of progression of the disease by reference to a correlation graph for each as shown in Figures 1 and 2.

As a further aspect of the invention we provide a product for prognosis of COPD severity in a patient which comprises means to report the concentration of soluble E-cadherin in a sample of blood serum taken from the patient.

We also provide a product for the prognosis of COPD severity in a patient which comprises means to report the concentration of soluble E-cadherin in a sample of urine taken from the patient.

For blood serum analysis, a 20-30μl volume of blood taken from a 'pin-prick' would be suitable and for urine analysis a sample of approximately 1ml taken "mid-flow" would be suitable.

sE-cadherin concentration may be measured using a commercially available kit from Takara. This kit allows the measurement of sE-cadherin, using standard ELISA technology and the standard curve provided, which allows interpretation of the measurement in terms of a concentration.

Example 1

Blood serum, urine and induced sputum from 4 patient groups (healthy non-smokers, healthy smokers, asthmatics and COPD patients) were sampled and the soluble E-cadherin concentration in each body fluid was measured.

FEV1 was measured using the method given above. Predicted (normal) FEV1 was calculated for each patient in accordance with the algorithm given in the above mentioned Morris et al (1971) paper and the actual FEV1 given as a percentage of predicted.

Table 1 contains information relating to all patients used in this example.

Pack years refers to the level of smoke exposure. One pack year equates to 20 cigarettes smoked per day for 1 year.
The medicaments used in the table refer to 'salb': salbutamol and 'atro': Atrovent (ipratropium bromide).

Table 1

Patient Group		Mean [sE-cadherin] in sputum supernatant (ng/ml)	Mean [sE-cadherin] in serum (ng/ml)	Mean [sE-cadherin] in urine (ng/ml)	Mean [IL-8]	Mean [creatinine] (mmol/L)	[Urine]/ [Creatinine] ratio	Age of patient	Sex of patient	Medication taken by patient	Pack Years	FEV1 % pred
COPD	1	294	5082	1795	5049	8.1	222	59	F	nil	35	60
COPD	2	94	4728	1518	10661	1.3	1168	66	M	salb, atro	44	66.2
COPD	3	810	6239	1900	3218	4.7	404	48	M	nil	30	64.5
COPD	4	71	7297	255	3598	0.6	425	45	M	nil	30	75
COPD	5	830	5342	3066	548	17.5	175	47	M	atro	25 (ex)	41
COPD	6	1749	6781	3603	4809	12.4	291	43	M	nil	30	69
COPD	7	179	5208	996	15397	3.2	311	45	F	nil	30	67.1
COPD	8	821	1761	5617	14056	10.9	515	54	M	nil	40	23
COPD	9	815	5198	2340	11147	2.8	836	56	F	nil	40	40.4
COPD	10	658	2736	5034	2954	12.3	409	65	M	salb, atro	30 (ex)	54
Mean values of Patient Group		632	5037	2612	7144	7.4	476	53	7 M	-	35	56

Patient Group		Mean [sE-cadherin] in sputum supernatant (ng/ml)	Mean [sE-cadherin] in serum (ng/ml)	Mean [sE-cadherin] in urine (ng/ml)	Mean [IL-8]	Mean [creatinine] (mmol/L)	[Urine]/ [Creatinine] ratio	Age of patient	Sex of patient	Medication taken by patient	Pack Years	FEV1 % pred
Healthy Smokers	1	506	5612	1775	827	9.4	189	42	F	nil	15	93
Healthy Smokers	2	547	5740	4568	221	15	304	42	F	nil	22	92
Healthy Smokers	3	376	4830	4310	796	17.5	246	38	F	nil	10	98
Healthy Smokers	4	463	5590	2609	716	4.4	593	35	F	nil	15	100
Healthy Smokers	5	446	5103	-	544	-	-	48	F	nil	20	90
Healthy Smokers	6	837	4733	878	461	16	55	26	F	nil	10	101
Healthy Smokers	7	1671	2761	780	3362	3.5	223	24	F	nil	11	105
Healthy Smokers	8	697	4856	1956	673	7	279	33	F	nil	15	98
Healthy Smokers	9	368	5585	1684	393	12.8	132	28	M	nil	10	109
Healthy Smokers	10	403	3431	9558	425	12.7	753	38	F	nil	20	93
Mean values of Patient Group		516	4824	3270	562	11.4	312	35	1 M	-	14.8	98

Patient Group		Mean [sE-cadherin] in sputum supernatant (ng/ml)	Mean [sE-cadherin] in serum (ng/ml)	Mean [sE-cadherin] in urine (ng/ml)	Mean [IL-8]	Mean [creatinine] (mmol/L)	[Urine]/ [Creatinine] ratio	Age of patient	Sex of patient	Medication taken by patient	Pack Years	FEV1 % pred
Healthy Non-Smokers	1	673	5951	842	704	7.4	114	28	F	nil	nil	101
Healthy Non-Smokers	2	1006	7463	3976	179	15	265	41	F	nil	nil	96
Healthy Non-Smokers	3	654	2617	750	547	3	250	28	F	nil	nil	99.4
Healthy Non-Smokers	4	538	4697	2737	603	8	342	31	F	nil	nil	97
Healthy Non-Smokers	5	1118	6804	4022	206	17.3	232	33	F	nil	nil	83
Healthy Non-Smokers	6	367	3544	3329	337	11.2	297	21	F	nil	nil	104
Healthy Non-Smokers	7	782	8697	4639	667	12.4	374	52	F	nil	nil	100
Healthy Non-Smokers	8	673	7357	4918	347	11.5	428	47	F	nil	nil	95
Healthy Non-Smokers	9	976	4041	4963	683	8.5	584	43	F	nil	nil	84
Healthy Non-Smokers	10	1375	6094	2199	584	20	110	28	F	nil	nil	99.4
Mean values of Patient Group		816	5726	3237	486	11.4	300	35	0 M	-	-	95.9

Patient Group		Mean [sE-cadherin] in sputum supernatant (ng/ml)	Mean [sE-cadherin] in serum (ng/ml)	Mean [sE-cadherin] in urine (ng/ml)	Mean [IL-8]	Mean [creatinine] (mmol/L)	[Urine]/ [Creatinine] ratio	Age of patient	Sex of patient	Medication taken by patient	Pack Years	FEV1 % pred
Asthma	1	334	5041	4900	753	13.7	358	39	M	salb	nil	101
Asthma	2	508	2027	3464	48	23	151	30	M	salb	nil	98
Asthma	3	240	6153	1817	73	7.1	256	22	F	salb	nil	89
Asthma	4	348	4168	1190	37	4.1	290	41	F	salb	nil	95
Asthma	5	577	5403	2228	528	10.3	216	27	M	salb	nil	94
Asthma	6	584	4625	4003	539	11	364	27	M	salb	nil	92
Asthma	7	320	5101	3406	285	16.7	204	33	F	salb	nil	84
Asthma	8	324	6190	320	496	-	-	40	F	salb	nil	98
Asthma	9	1767	4044	8039	2070	6.4	1256	37	M	salb	nil	89
Asthma	10	693	6038	2034	125	8.5	239	21	F	salb	Nil	98
Mean values of Patient Group		436	4879	3140	320	11.2	370	32	5 M	-	-	94

The results are shown in the following Figures:

Figure 1 - FEV1 (as a percentage of the predicted value) as a function of concentration of soluble E-cadherin in blood serum

Figure 2 - FEV1 (as a percentage of the predicted value) as a function of concentration of soluble E-cadherin in urine.

The predicted value of FEV1 was determined according to Morris JF et al 1971: Am Rev Resp Dis **103,** 57-67.

The results presented in Figure 1 show that FEV1 (as a percentage of the predicted value) (y) is correlated with concentration of soluble E-cadherin in blood serum (x) in COPD patients according to Spearman's rank correlation analysis.

The correlation coefficient and p-values for the 4 patient groups from these data are as follows:

	Corr coeff	p-value
Healthy non-smokers	-0.36	0.521
Healthy smokers	-0.23	0.307
Asthmatics	0.02	0.946
COPD patients	0.67	0.033

The results presented in Figure 2 show that FEV1 (as a percentage of the predicted value) (y) is correlated with concentration of soluble E-cadherin in urine (x) in COPD patients according to Spearman's rank correlation analysis.

The correlation coefficient and p-values for the 4 patient groups from these data are as follows:

	corr. coeff.	p-value
COPD	-0.66	0.038
Healthy Smokers	-0.76	0.016
Healthy Non-Smokers	-0.57	0.088
Asthma	-0.11	0.761

Both Figures 1 and 2 show that there is no correlation between FEV1 and concentration of soluble E-cadherin in urine or blood serum in asthmatics.

Figure 1

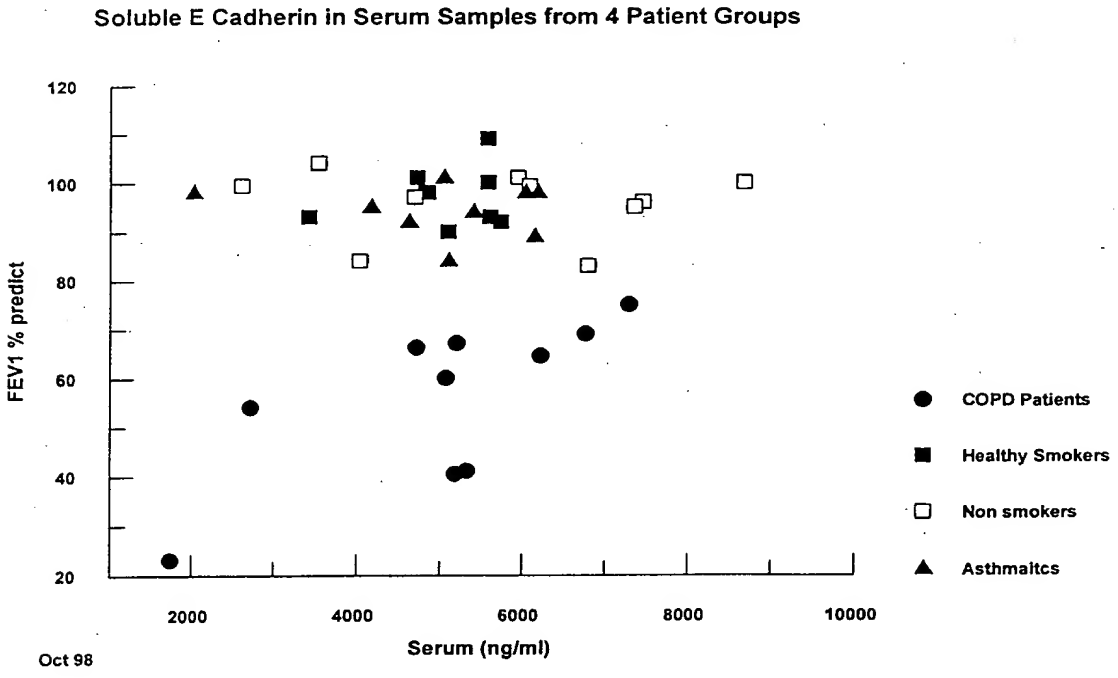
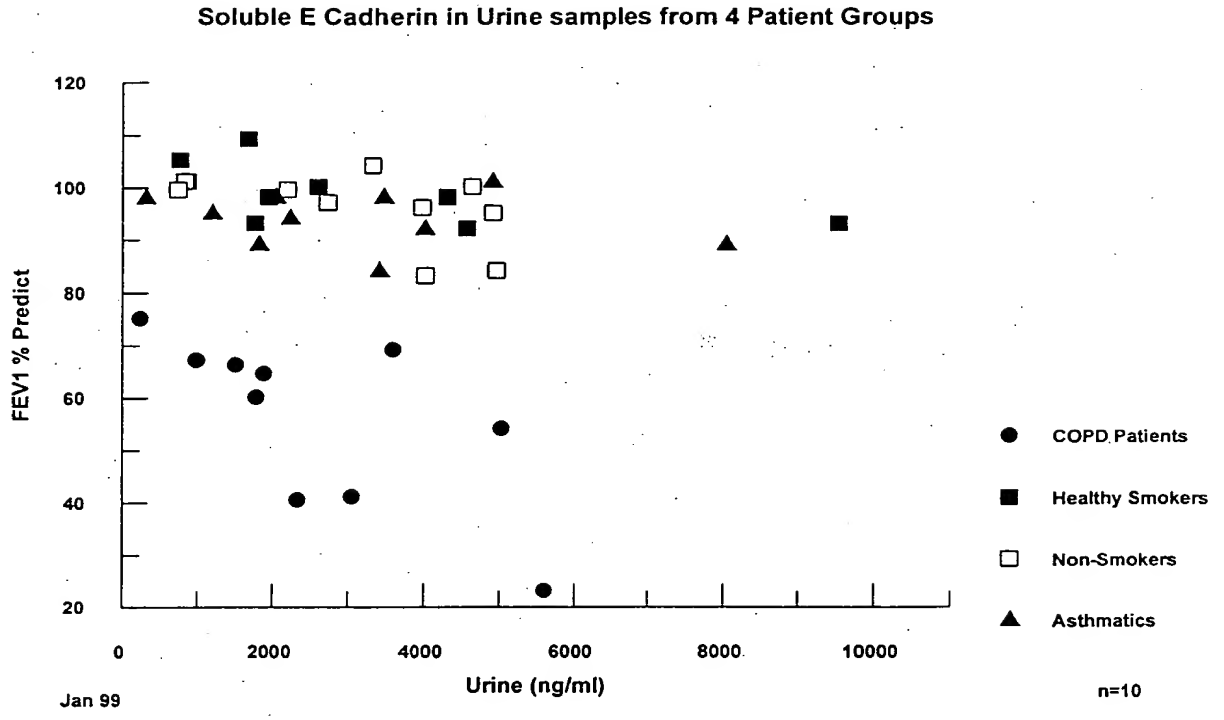


Figure 2



The application of which this description and claims forms part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application may be directed to any feature or combination of features described herein. They may take the form of product, composition, process, or use claims and may include, by way of example and without limitation, the following claims:

Claims

1. A method of determining the severity of COPD in a patient which comprises measuring the concentration of soluble E-cadherin in the patient's urine and determining the extent of severity by reference to a correlation graph as shown in Figure 1.

2 A method of determining the severity of COPD in a patient which comprises measuring the concentration of soluble E-cadherin in the patient's blood serum and determining the extent of severity by reference to a correlation graph as shown in Figure 2.

3 A method according to claims 1 and 2 which comprises measuring the concentration of soluble E-cadherin in the patient's blood serum and urine and determining the extent of severity by reference to a correlation graph for each as shown in Figures 1 and 2.

4 Use of soluble E-cadherin in the manufacture of a prognostic product for determination of COPD disease severity in a patient.

5. A method of determining the responsiveness of a patient with COPD to therapy which comprises monitoring the concentration of soluble E-cadherin in the blood serum of the patient with time and determining the rate of change of extent of progression of the disease by reference to a correlation graph as shown in Figure 1.

6 A method of determining the responsiveness of a patient with COPD to therapy which comprises monitoring the concentration of soluble E-cadherin in the urine of the patient with time and determining the rate of change of

extent of progression of the disease by reference to a correlation graph as shown in Figure 2.

7 A method according to claim 5 or claim 6 which comprises monitoring the concentration of soluble E-cadherin in the urine and blood serum of the patient with time and determining the rate of change of extent of progression of the disease by reference to a correlation graph for each as shown in Figures 1 and 2.

8 A product for the prognosis of COPD severity in a patient which comprises means to report the concentration of soluble E-cadherin in a sample of blood serum taken from the patient.

9 A product for the prognosis of COPD severity in a patient which comprises means to report the concentration of soluble E-cadherin in a sample of urine taken from the patient.